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(54) Title: METHODS OF TREATMENT

(57) Abstract

A method of treating weight loss due to underlying disease in a patient, the method comprising administering to the patient an effective amount of an agent which reduces sympathetic nervous system activity. A method of treating weight loss due to underlying disease in a patient, the method comprising administering to the patient an effective amount of any one or more of the following: a compound which inhibits the effect of aldosterone such as an aldosterone antagonist; a chymase inhibitor; a cathepsin B inhibitor; a β receptor blocker; an imidazoline receptor antagonist; a centrally acting α receptor antagonist; a peripherally acting α receptor antagonist; a ganglion blocking agent; a drug that has an effect on cardiovascular reflexes and thereby reduces SNS activity such as an opiate; scopolamine; an endothelin receptor antogonist; and a xanthine oxidase inhibitor. The methods are particularly useful in treating cardiac cachexia.

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METHODS OF TREATMENT

The present invention relates to methods of treatment, in particular it relates to methods of treating weight loss due to underlying disease (cachexia).

Weight loss due to underlying disease, often termed "cachexia", occurs in patients with a wide variety of diseases including acquired immune deficiency syndrome (AIDS), liver cirrhosis, chronic obstructive pulmonary disease, chronic renal failure, chronic infections including pneumonia, cancer (cancer cachexia), diabetes and heart disease including hypertension and chronic heart failure (CHF) (cardiac cachexia). Cachexia may also occur idiopathically.

In all cases, cachexia may be an indicator of a poor prognosis and its reversal, stopping or at least slowing down, is desirable. Indeed, a strong relationship between weight loss and mortality has been found for many conditions.

Hormonal changes and catabolic/anabolic imbalance in chronic heart failure (CHF) and their relevance in cardiac cachexia has been discussed in Anker *et al* (1997) *Circulation* **96**, 526-534. Similarly, catecholamine levels, serum uric acid levels, TNFα levels and other hormone levels have been measured in patients with CHF (see, for example, Anker *et al* (1997) *Heart* **78**, 39-43; Anker *et al* (1998) *Q J. Med.* **91**, 199-203; Anker (1998) *Eur. Heart J.* **19**, (Suppl F), F56-F61; Anker *et al* (1997) *J. Amer. Coll. Cardiol.* **30**, 997-1001; Anker *et al* (1999) *Eur. Heart J.* **20**, 683-693; Anker (1999) *Chest* **115**, 836-847). In addition, studies have been made

of the loss of bone mineral in patients with cachexia due to CHF (Anker *et al* (1999) *Am. J. Cardiol.* **83**, 612-615).

However, no-one has suggested that reducing sympathetic nervous system activity and/or improving cardiovascular reflex status would be beneficial to patients with cardiac cachexia and also to patients with cachexia due to any cause and, indeed, idiopathic cachexia.

A first aspect of the invention provides a method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of an agent which reduces sympathetic nervous system activity and/or improves cardiovascular reflex status.

Without prejudice to further aspects of the invention and without being bound by any theories as to how the invention works, we believe that at least some of the information described in the Examples indicates that agents which inhibit sympathetic nervous system activity, either directly or indirectly, (for example by directly or indirectly having ergo-reflex, chemoreflex or baroreflex effects) have a beneficial effect on cachexia probably by a reduction of apoptosis, a reduction in metabolic rates or by vasodilation with better blood flow to tissues. We provide information that, surprisingly, certain pathways are abnormal in cachexia due to a wide range of underlying diseases, but they are not abnormal in weight loss due to starvation.

A second aspect of the invention provides a method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of any one or more of the following: a

compound which inhibits the effect of aldosterone such as an aldosterone antagonist; a chymase inhibitor; a cathepsin inhibitor; a β receptor blocker; an imidazoline receptor antagonist; a centrally acting α receptor antagonist; a peripherally acting α receptor antagonist; a ganglion blocking agent; a drug that has an effect on cardiovascular reflexes and thereby reduce SNS activity such as an opiate *via* chemoreceptor; scopolamine; an endothelin receptor antagonist; a xanthine oxidase inhibitor; and erythropoietin.

The method may be used on any mammal and so the term "patient" includes a human patient and also includes any other mammal including domestic animals such as cats and dogs, and farm animals such as cows, pigs, horses, sheep, goats and the like. It is preferred if the method is used to treat humans.

A third aspect of the invention provides a method of treating weight loss due to underlying disease in a patient the method comprising electrically stimulating the patient's muscles. This may be done using any transcutaneous electrical stimulator applied to the skin over a muscle or its nerve to stimulate muscle contractions. Suitably, to increase muscle strength and bulk high frequency stimulation (eg 50 Hz) is used. In contrast low frequency stimulation (eg 10 Hz) may enhance slow fatigue resistant fibres and could cause a fibre type shift which could reduce strength and so is not preferred.

In treating weight loss due to underlying disease in a patient it is useful if the weight loss is reversed or stopped or at least slowed down.

The aforementioned compounds and procedures are useful for the treatment or prevention of weight loss due to underlying disease (cachexia). These underlying diseases include, for example, but are not restricted to, AIDS, liver cirrhosis, chronic obstructive pulmonary disease with or without emphysema, chronic renal failure, chronic infections (like pneumonia), cancer (ie cancer cachexia), and heart disease including hypertension and chronic heart failure (ie cardiac cachexia), and idiopathic cachexia (ie cachexia due to unknown disease).

Compounds or procedures that may reduce angiotensin II plasma levels and therefore are useful in the practice of the invention include:

1. any compound with an inhibiting effect on aldosterone, eg aldosterone antagonists such as spironolactone (which may be given at between 12.5 mg and 300 mg per day, orally) and testolactone (which may be given at 40 mg/kg per day, orally), RU40555 (which may be given at 10-30 mg/kg orally), RU26752 (a synthetic aldosterone antagonist), canrenoate (which may be given at 20 mg/day iv) also known as Canrenoate Potassium, eplerenone (oral), 3-(17 beta-hydroxy-3-oxoandrosta-1,4,6,11-tetraen-17 alpha-yl)propionic acid gamma-lactone, 3-(9 alpha-fluoro-17 beta-hydroxy-3-oxoandrost-4-en-17 alpha-yl)propionic acid gamma-lactone (31), dihydrospirorenone, spirorenone, 15,16-methylene derivatives of spironolactone, mespirenone (CAS 87952-98-5) and SC9420;
2. chymase inhibitors, including alendronate, aprotinin and tissue inhibitors of matrix metalloproteinases (TIMPs);

3. cathepsin B inhibitors, including epoxysuccinyl peptides such as CA-074 and E-64c, stefinA, cystatin C (endogenous inhibitor), CA074 (a specific inhibitor of cathepsin B) and E-64 (natural inhibitor of cathepsin B);
4. exercise training;
5. electrical muscle stimulation;

Compounds that may reduce catecholamine plasma levels and the activity of the sympathetic nervous system (SNS) include:

6. Beta (β) receptor blockers including acebutolol, alprenolol, atenolol, betaxolol, bisoprolol, carteolol, celiprolol, esmolol, labetolol, lavobunolol, metipranolol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, propanolol, sotalol, nebivolol, carvedilol, bucindolol and timolol; Atenolol and bisoprolol are preferred.
7. imidazoline receptor antagonists (including moxonidine, clonidine, rilmenidine, pentamidine (1,5-bis (4-amidonophenoxy)pentane) and alpha methyl dopa;
8. centrally acting alpha receptor agonists like clonidine;
9. peripherally acting alpha receptor antagonists such as doxazosin (which may be given at 1-16 mg orally per day), prazosin, terazosin and ipsapirone;
10. ganglion blocking agents including azamethonium, dicolinium, hexamethonium, mecamylamine, pentamethonium, pentolinium, trimetaphan, benzohexonium, hexafluorenium, cypenam, trimethaphan canfosulfonate, tetraethylammonium bromide, and

synapleg;

11. drugs that have effects on cardiovascular reflexes and thereby reduce SNS activity including
    - opiates (*via* chemoreceptor) such as dihydrocodeine, morphine, diamorphine and buprenorphine
    - scopolamine;
12. xanthine oxidase inhibitors including allopurinol (which may be given at 50-1000 mg per day orally), 7,8-dihydroneopterin, 5,6,7,8-tetrahydrobiopterin, leukopterin, xanthopterin, neopterin, biopterin, 4-amino-6-hydroxypyrazolo[3,4-d]pyrimidine (AHPP), and oxypurinol;

Allopurinol is preferred.

Endothelin receptor (such as ET-I receptor) antagonists include

- endothelin receptor A antagonist BQ 123
- ETB-receptor antagonist BQ-788
- A-216546 ([2S-(2,2-dimethylpentyl)-4S-(7-methoxy-1,3-benzodioxol-5-yl)-1-(N,N-di(n-butyl) aminocarbonylmethyl)-pyrrolidine-3R-carboxylic acid), a potent antagonist with > 25,000-fold selectivity for the endothelin ET(A) receptor
- ABT-627 (1, A-147627, active enantiomer of A-127722), a 2,4-diaryl substituted pyrrolidine-3-carboxylic acid based endothelin receptor-A antagonist. This compound binds to the ETA receptor with an affinity (Ki) of 0.034 nM and with a 2000-fold selectivity for the ETA receptor versus the ETB receptor.
- IRL 3461: a potent endothelin antagonist with balanced ETA/ETB

affinity

- oral endothelin-receptor antagonist bosentan (0.1 - 1.0 g BID, preferred 0.25 - 0.5 g BID), has combined ETA/ETB affinity
- LU135252, a selective antagonist of the ETA receptor
- S-0139, (+)-disodium 27-[(E)-3-[2-[(E)-3-carboxylatoacryloylamino]-5-hydroxyphenl]acrylayloxy]-3-oxoolean-12-en-28-oate, an ETA selective antagonist
- N-(6-(2-(5-bromopyrimidin-4-yl)-4-(2-hydroxy-1, 1-dimethylethyl)-benzensulfonamide sodium salt sesquihydrate (T-0201), a nonpeptide endothelin (ET) receptor antagonist. In binding studies, T-0201 competitively antagonized the specific binding of [125I]-ET-1 to human cloned ETA receptors
- unselective ET(A)/ET(B) receptor antagonist, PD 142,893
- PD164333, an analogue of the orally active butenolide antagonists of the endothelin ETA receptor
- Ro 61-1790 [5-methyl-pyridine-2-sulfonic acid 6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2-(2-1H-tetrazol-5-yl-+++pyridin-4-yl)-pyrimidin-4-ylamide] is a competitive ET antagonist with an affinity to ETA receptor in the subnanomolar range. It has an approximately 1000-fold selectivity for the ETA vs the ETB receptor
- ET-A antagonist PD-156,707
- SB 209670, a rationally designed potent nonpeptide endothelin receptor antagonist
- endothelin B receptor-selective antagonist: IRL 1038, [Cys11-Cys15]-endothelin-1(11-21)
- WS-7338 B, a specific antagonist for vascular ETA receptors.

The endothelins (ETs) are a family of bicyclic 21-amino acid peptides that are potent and prolonged vasoconstrictors. ET receptor antagonists improve peripheral blood flow, improve muscle metabolic status and thereby ergoreflex, and, we believe, thereby reduce SNS activity. ET-A receptor blockade is preferred in the practice of the invention.

Various compounds are described in at least the following publications:

RU40555

Evaluation of RU28318 and RU40555 as selective mineralocorticoid receptor and glucocorticoid receptor antagonists, respectively: receptor measures and functional studies. Kim PJ, Cole MA, Kalman BA, Spencer RL. *J Steroid Biochem Mol Biol* 1998 Nov 67:3 213-22.

RU 26752

Effects of antimineralocorticoid RU 26752 on steroid-induced hypertension in rats. Kalimi M, Opoku J, Agarwal M, Corley K. *Am J Physiol* 1990 May 258:5 Pt 1 E737-9.

CAS 87952-98-5

Inhibitory effects of the novel anti-aldosterone compound mespirenone on adrenocortical steroidogenesis *in vitro*. Weindel K, Lewicka S, Vecsei P. *Arzneimittelforschung* 1991 Sep 41:9 946-9.

SC9420

Blocking by spironolactone (SC 9420) of the action of aldosterone upon the intestinal transport of potassium, sodium, and water. Elmslie RG, Mulholland AT, Shields R. *Gut* 1966 Dec 7:6 697-9.

TIMPS

Bimolecular interaction of matrix metalloproteinases and their inhibitors TIMPs. Tschesche H. *J Protein Chem* 1998 Aug 17:6 549-51.

CA-074

Novel epoxysuccinyl peptides. A selective inhibitor of cathepsin B, in vivo. Towatari T, Nikawa T, Murata M, Yokoo C, Tamai M, Hanada K, Katunuma N. *FEBS Lett* 1991 Mar 25 280:2 311-5.

E-64c

Effects of selective inhibition of cathepsin B and general inhibition of cysteine proteinases on lysosomal proteolysis in rat liver *in vivo* and *in vitro*. Ohshita T, Nikawa T, Towatari T, Katunuma N. *Eur J Biochem* 1992 Oct 1 209:1 223-31.

Stefin A

Identification of bovine stefin A, a novel protein inhibitor of cysteine proteinases. Turk B, Ritonja A, Björk I, Stoka V, Dolenc I, Turk V. *FEBS Lett* 1995 Feb 27 360:2 101-5.

cystatin C

Two-step mechanism of inhibition of cathepsin B by cystatin C due to displacement of the proteinase occluding loop. Nycander M, Estrada S, Mort JS, Abrahamson M, Björk I. *FEBS Lett* 1998 Jan 23 422:1 61-4.

E64

Inhibitions by E-64 derivatives of rat liver cathepsin B and cathepsin L *in vitro* and *in vivo*. Hashida S, Towatari T, Kominami E, Katunuma N. J Biochem (Tokyo) 1980 Dec 88:6 1805-11.

BQ 123

In vitro biological profile of a highly potent novel endothelin (ET) antagonist BQ-123 selective for the ETA receptor. Ihara M, Ishikawa K, Fukuroda T, Saeki T, Funabashi K, Fukami T, Suda H, Yano M. *J Cardiovasc Pharmacol* 1992 20 Suppl 12 S11-4.

BQ-788

Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. Ishikawa K, Ihara M, Noguchi K, Mase T, Mino N, Saeki T, Fukuroda T, Fukami T, Ozaki S, Nagase T, *et al. Proc Natl Acad Sci U S A* 1994 May 24 91:11 4892-6.

A-216546

Pyrrolidine-3-carboxylic acids as endothelin antagonists. 3. Discovery of a potent, 2-nonaryl, highly selective ETA antagonist (A-216546). Liu G, Henry KJ Jr, Szczepankiewicz BG, Winn M, Kozmina NS, Boyd SA, Wasicak J, von Geldern TW, Wu-Wong JR, Chiou WJ, Dixon DB,

Nguyen B, Marsh KC, Opgenorth TJ. *J Med Chem* 1998 Aug 13 41:17 3261-75.

A-127722

Potent and selective non-benzodioxole-containing endothelin-A receptor antagonists. Tasker AS, Sorensen BK, Jae HS, Winn M, von Geldern TW, Dixon DB, Chiou WJ, Dayton BD, Calzadila S, Hernandez L, Marsh KC, WuWong JR, Opgenorth TJ. *J Med Chem* 1997 Jan 31 40:3 322-30.

ABT-627

Pyrrolidine-3-carboxylic acids as endothelin antagonists. 2. Sulfonamide-based ETA/ETB mixed antagonists. Jae HS, Winn M, Dixon DB, Marsh KC, Nguyen B, Opgenorth TJ, von Geldern TW. *J Med Chem* 1997 Sep 26 40:20 3217-27.

IRL 3461

Discovery of IRL 3461: a novel and potent endothelin antagonist with balanced ETA/ETB affinity. Sakaki J, Murata T, Yuumoto Y, Nakamura I, Trueh T, Pitterna T, Iwasaki G, Oda K, Yamamura T, Hayakawa K. *Bioorg Med Chem Lett* 1998 Aug 18 8:16 2241-6.

LU135252

Effects of chronic ETA-receptor blockade in angiotensin II-induced hypertension. d'Uscio LV, Moreau P, Shaw S, Takase H, Barton M, Lüscher TF. *Hypertension* 1997 Jan 29:1 Pt 2 435-41.

S-0139

Binding characterization of [3H]S-0139, an antagonist of the endothelin ET(A) receptor subtype. Mihara S, Tozawa F, Itazaki K, Fujimoto M. *Eur J Pharmacol* 1998 Jan 26 342:2-3 319-24.

T-0201

Pharmacological profile of T-0201, a highly potent and orally active endothelin receptor antagonist. Hoshino T, Yamauchi R, Kikkawa K, Yabana H, Murata S. *Pharmacol Exp Ther* 1998 Aug 286:2 643-9.

PD 142,893

In vitro and *in vivo* studies with a series of hexapeptide endothelin antagonists. Doherty AM, Cody WL, He JX, DePue PL, Cheng XM, Welch KM, Flynn MA, Reynolds EE, LaDouceur DM, Davis LS, *et al.* *J Cardiovasc Pharmacol* 1993 22 Suppl 8 S98-102.

PD164333

Characterization of [^{125}I]-PD164333, an ETA selective non-peptide radiolabelled antagonist, in normal and diseased human tissues. Davenport AP, Kuc RE, Ashby MJ, Patt WC, Doherty AM. *Br J Pharmacol* 1998 Jan 123:2 223-30.

Ro 61-1790

Ro 61-1790, a new hydrosoluble endothelin antagonist: general pharmacology and effects on experimental cerebral vasospasm. Roux S, Breu V, Giller T, Neidhart W, Ramuz H, Coassolo P, Clozel JP, Clozel M. *J Pharmacol Exp Ther* 1997 Dec 283:3 1110-8.

PD 156707

Affinity and selectivity of PD156707, a novel nonpeptide endothelin antagonist, for human ET(A) and ET(B) receptors. Maguire JJ, Kuc RE, Davenport AP. *J Pharmacol Exp Ther* 1997 Feb 280:2 1102-8.

SB209670

Nonpeptide endothelin receptor antagonists. I. Effects on binding and signal transduction on human endothelinA and endothelinB receptors. Nambi P, Elshourbagy N, Wu HL, Pullen M, Ohlstein EH, Brooks DP, Lago MA, Elliott JD, Gleason JG, Ruffolo RR Jr. *J Pharmacol Exp Ther* 1994 Nov 271:2 755-61.

WS-7338

WS-7338, new endothelin receptor antagonists isolated from Streptomyces sp. No. 7338. II. Biological characterization and pharmacological characterization of WS-7338 B. Miyata S, Hashimoto M, Fujie K, Nishikawa M, Kiyoto S, Okuhara M, Kohsaka M. *J Antibiot (Tokyo)* 1992 Jan 45:1 83-7.

Erythropoietin may be any suitable form of erythropoietin. Typically, when the patient to be treated is a human, the erythropoietin is recombinant human erythropoietin (rhEPO).

Without prejudice to any aspect of the invention, and without being bound by any theory concerning the way the invention works, we believe that EPO improves oxygen delivery to muscle which leads to a better muscle metabolic state which decrease ergoreflex and improves cachexia.

Without prejudice to any aspect of the invention and without being bound by any theory concerning the way the invention works, we believe that administration of opiate agents will suppress firing of the arterial chemoreflexes and *via* this action will inhibit sympathetic nervous system activity and *via* this action will delay the progression of cachexia.

Without prejudice to any aspect of the invention, and without being bound by any theory concerning the way the invention works, we believe that scopolamine enhances baroreflex activity and by specific enhancement of vagal activity will *via* this action inhibit sympathetic nervous system activity and *via* this action will delay the progression of cachexia.

Without prejudice to any aspect of the invention, and without being bound by any theory concerning the way the invention works, we believe that aldosterone antagonists may present or reduce myocardial and skeletal muscle fibrosis which enables muscle to act more efficiently and thereby prevent or reduce the stimulus for SNS reflex abnormalities.

The above-mentioned classes of compounds and procedures are also useful in the treatment or prevention of weight loss due to the ageing process. They, as well as others mentioned below, are also useful in the enhancement of exercise performance in health.

Thus, a fourth aspect of the invention provides a method of treating or preventing weight loss due to the ageing process in a patient the method comprising administering to the patient an effective amount of an agent which reduces sympathetic nervous system activity.

A fifth aspect of the invention provides a method of treating or preventing weight loss due to the ageing process in a patient the method comprising administering to the patient an effective amount of any one or more of a compound which inhibits the effect of aldosterone such as an aldosterone antagonist; a chymase inhibitor; a cathepsin inhibitor; a β receptor blocker; an imidazoline receptor antagonist; a centrally acting α receptor antagonist; a peripherally acting α receptor antagonist; a ganglion blocking agent; a drug that has an effect on cardiovascular reflexes and thereby reduce SNS activity such as an opiate *via* chemoreceptor, a digitalis alkaloid *via* enhancement of baroreflex sensitivity; scopolamine; an ET-1 receptor antagonist; an xanthine oxidase inhibitor; and erythropoietin.

Without prejudice to any aspect of the invention, and without being bound by any theory concerning the way the invention works, we believe that digitalis alkaloids will, *via* increasing sensitivity of the arterial baroreflexes, inhibit sympathetic nervous system activity and, by this action, delay the weight loss.

A sixth aspect of the invention provides a method of treating or preventing weight loss due to the ageing process in a patient the method comprising electrically stimulating the patient's muscles. Typically, the patient to be treated is >65 years old. An overview about human weight homeostasis and weight loss due to ageing is given in Anker *et al* (1999) *Chest* **115**, 836-847.

A seventh aspect of the invention provides a method of enhancing exercise performance in a healthy individual the method comprising administering to the individual an effective amount of any one or more of a compound

which inhibits the effect of aldosterone such as an aldosterone antagonist; a chymase inhibitor; a cathepsin inhibitor; a β receptor blocker; an imidazoline receptor antagonist; a centrally acting α receptor antagonist; a peripherally acting α receptor antagonist; a ganglion blocking agent; a drug that has an effect on cardiovascular reflexes and thereby reduce SNS activity such as an opiate *via* chemoreceptor, a digitalis alkaloid *via* enhancement of baroreflex sensitivity, scopolamine, or an anabolic growth factor like growth hormone and insulin-like growth factor-I (IGF-I) *via* effects on metabo-ergoreceptor; an ET-1 receptor antagonist; a TNFα antagonist; an xanthine oxidase inhibitor; and erythropoietin, and an eighth aspect of the invention provides a method of enhancing exercise performance in a healthy patient the method comprising electrically stimulating the patient's muscles.

Similarly, without prejudice and without being bound by any theory, we believe that anabolic growth factors and insulin growth factor-1 may increase skeletal muscle bulk and reduce the metabolic stress in a given muscle on exercise which will produce less stimulation of the work-sensitive muscle ergoreceptors (metaboreceptors) and will *via* this action inhibit sympathetic nervous system activity and *via* this action will delay the progression of cachexia.

Suitable digitalis alkaloids include digoxin and digitoxin and are believed to work in the context of the invention *via* enhancement of baroreflex sensitivity.

Suitable anabolic growth factors include growth hormone and insulin-like growth factor-I, and are believed to act via effects on the metabo-

ergoreceptor.

By "TNFα antagonists" we mean any agent which blocks the activity of TNFα. Such antagonists include anti-TNFα antibodies and suitable forms of TNFα receptor (eg soluble forms) that bind to TNFα and render TNFα molecules to be biologically less active.

Furthermore, the classes of compounds described in numbered groups 1, and 6 to 10 are also useful in preventing weight loss consequent to cardiovascular disorders in patients at risk of heart disease including hypertension, dyslipidaemia and diabetes.

Thus, a ninth aspect of the invention provides a method of preventing weight loss consequent to a cardiovascular disorder in a patient at risk of heart disease the method comprising administering to the patient an effective amount of any one or more of a compound with an inhibiting effect on aldosterone; a β-receptor blocker; an imidazoline receptor antagonist; a centrally acting α receptor agonist, a peripherally acting α receptor antagonist; and a ganglion blocking agent.

The drugs are administered to the patient in any suitable form or by any suitable route in order to have the desired effect. The invention also includes the use of the drug in the manufacture of a medicament for treating the patient as said.

The aforementioned compounds for use in the methods of the invention or a formulation thereof may be administered by any conventional method including oral and parenteral (eg subcutaneous or intramuscular or

intravenous) injection and inhaled and per-rectal and buccal. The treatment may consist of a single dose or a plurality of doses over a period of time.

Whilst it is possible for a compound for use in the methods of the invention to be administered alone, it is preferable to present it as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free.

As noted above, the compounds for use in the methods of the invention may be formulated for use. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient (compound of the invention) with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (eg sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide desired release profile.

Formulations suitable for parenteral including intravenous administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

Typically, the drug is administered when cachexia is diagnosed (duration of treatment: for the lifetime of the patient) or if a patient is thought to be at risk of developing cachexia. The drug is administered at a frequency and in sufficient amount to maintain trough levels of the agent at about 50% of peak dosing levels.

Other drugs which may be suitable in the practice of the invention as discussed above are known in the art; some of these compounds are listed for example in the latest editions of the British National Formulary and in the latest edition of Martindale's Pharmacoepia.

The invention will now be described in more detail with reference to the following Examples and Figures wherein

Figure 1 shows individual data for noradrenaline plasma levels which is summarised in Figure 2.

Figure 2 shows that chronic wasting disorders show increased activity of SNS (sympathetic nervous system) as evidenced by increased plasma

noradrenaline levels. All of the cachectic disorders marked (*) have mean plasma noradrenaline levels which are higher than normal.

Figure 3 shows that, on average, patients with active wasting disease have 2.5 to 13-fold increased aldosterone levels compared to healthy controls (their mean : 43.2 ng/ml, upper limit or normal : 81 ng/ml). Patients with weight loss due to malnutrition have normal aldosterone levels.

Figure 4 shows that patients with wasting disease have increased angiotensin II plasma levels.

Figure 5 shows that the frequency of developing cardiac cachexia over time is lower in patients treated with enalapril compared to patients treated with placebo.

Example 1: Catecholamines in chronic heart failure patients

Noradrenaline plasma levels in chronic heart failure patients

Chronic heart failure (CHF) is a complex disorder affecting an increasing number of patients in the community with a prevalence of 10 to 30% in people over the age of 65 years [Cowie MR, Mostered AA, Wood DA, Deckers JW, Poole-Wilson PA, Sutton GC, Grobbee DE. The epidemiology of heart failure. *Europ Heart J* 1997; **18**:208-225.]. Multiple physiological pathways are pathologically affected, and a series of vicious cycles have been suggested that could transform cardiac abnormalities into haemodynamic, endocrine, immunological, and

muscular abnormalities that all contribute to the clinical picture of chronic heart failure [Packer M. The neurohormonal hypothesis: A theory to explain the mechanism of disease progression in heart failure. *J Am Coll Cardiol* 1992;**20**:248-254; Anker SD, Clark AL, Kemp M, Salsbury C, Teixeira MM, Hellewell PG, Coats AJS. Tumor necrosis factor and steroid metabolism in chronic heart failure: possible relation to muscle wasting. *J Am Coll Cardiol* 1997; **30**:997-1001; Coats AJS, Clark AL, Piepoli M, Volterrani M, Poole-Wilson PA. Symptoms and quality of life in heart failure; the muscle hypothesis. *Br Heart J* 1994; **72**:S36-S39.]. One of the most studied aspects is activation of the sympathetic nervous system (SNS). Activation of the SNS can be expressed in several different ways. Apart from measuring circulating catecholamines (particularly noradrenaline, adrenaline, and dopamine), it is possible to assess sympathetic nervous excitation directly by measuring nerve impulses [Van de Borne P, Montano N, Zimmerman B, Pagani M, Somers VK. Relationship between repeated measures of hemodynamics, muscle sympathetic nerve activity, and their spectral oscillations. *Circulation* 1997; **96**:4326-4332.], or indirectly by analysing heart rate and blood pressure variability [Ponikowski P, Anker SD, Chua TP, Szelemej R, Piepoli M, Adamopoulos S, Webb-Peploe K, Harrington D, Banasiak W, Wrabec K, Coats AJS. Depressed heart rate variability as an independent predictor of death in patients with chronic heart failure. *Am J Cardiol* 1997;**79**:1645-1650]. The technique of assessing catecholamine levels has also been developed further by assessing the catecholamine spill-over using radio-labelled tracers [Coats Adamopoulos S, Radelli A, McCance A, Meyer TE, Bernardi L, Solda PL, Davey P, Ormerod O, Forfar C, Conway J, Sleight P. Controlled trial of physical training in chronic heart

failure: exercise performance, hemodynamics, ventilation, and autonomic function. *Circulation* 1992;**85**:2119-2131.]. Nevertheless, measurement of catecholamine levels at rest are the most widely used technique. In this respect it is important to note, that noradrenaline and adrenaline are not only released from the adrenal medulla (as hormones), but that they are also neurotransmitters that are released into the synaptic cleft of sympathetic post-ganglionic nerves (therefore also termed adrenergic). Only a small proportion of the synaptically released catecholamines spills over into the circulation. Therefore measured plasma concentrations of noradrenaline and adrenaline may in some circumstances grossly underestimate the local catecholamine concentration in the adrenergic synapses.

Catecholamines: from myocardial infarction to heart failure

Sympathetic activation is well recognised to be important contributing to the development of myocardial ischaemia [Heusch G. α-Adrenergic mechanisms in myocardial ischaemia. *Circulation* 1990;**81**:1-13.]. Cardiac β-receptors mediate increases of heart rate and inotropy, that under normal conditions lead to coronary dilation to match the oxygen demand. The direct effect of catecholamines on the coronary blood vessel is vasoconstriction mediated via α-adrenoreceptors [Berne RM. Effect of epinephrine and norepinephrine on coronary circulation. *Circ Res* 1958;**6**:644-655.]. During exercise catecholaminergic vasoconstriction is mainly mediated through circulating catecholamines and not through local hormone release [Chilian WM, Harrison DG, Haws CW, Snyder WD, Marcus ML. Adrenergic coronary tone during submaximal exercise in the

dog is produced by circulating catecholamines. Evidence for adrenergic denervation supersensitivity in the myocardium but not in coronary vessels. *Circ Res* 1986;**58**:68-82.]. After the development of coronary plaques and stenosis, the vasodilatory flow reserve is reduced and the metabolic vasodilation is more and more and more reduced as a result of α-adrenergic coronary vasoconstriction [Heusch G, Deussen A. The effects of cardiac sympathetic nerve stimulationon the perfusion of stenotic coronary arteries in the dog. *Circ Res* 1983;**53**:8-15.].

Dramatic increases of catecholamine levels have been detected early after the onset of infarction in a variety of studies. Alone between 1969 and 1980, 15 studies with about 25000 patients and 5000 control subjects (see overview in [Goldstein DS. Plasma noradrenaline as an indicator of sympathetic neutral activity in clinical cardiology. *Am J Cardiol* 1981;**48**:1147-1154.]) have investigated plasma noradrenaline levels after myocardial infarction. Catecholamine levels peak within minutes to few hours after the onset of symptoms, and they continue to be raised for several days. The degree of the enzymatic changes during the myocardial infarction [Vetter NJ, Adams W, Strange RC, Oliver MF. Initial metabolic and hormonal response to acute myocardial infarction. *Lancet* 1974;**1**:284-289.], ie severity of the heart attack, the early onset of ventricular arrhythmias [McDonald L, Baker C, Bray C, McDonald A, Restieaux N. Plasma-catecholamines after myocardial infarction. *Lancet* 1969;**2**:1021-1023.], the development of cardiogenic shock [Benedict CR, Grahame-Smith DG. Plasma adrenaline concentrations and dopamine-beta-hydrolase activity in myocardial infarction with and without cardiogenic shock. *Br Heart J* 1979;**42**:214-220.], and of congestive heart failure

[McDonald *et al* (1969) *Lancet* **2**:1021-1023; Siggers DCM, Salter C, Fluck DC. Serial plasma adrenaline and noradrenaline levels in myocardial infarction using a new double isotope technique. *Br Heart J* 1971;**33**:878-883.] are all related to plasma catecholamine levels. In patients with myocardial infarction and clinical heart failure noradrenaline remains elevated for about 1 month [Sigurdsson A, Held P, Swedberg K. Short- and long-term neurohormonal activation following acute myocardial infarction. *Am Heart J* 1993;**126**:1068-1076.]. Sedative treatment with morphines [Mueller HS, Gory DJ, Rao PS, Mudd G, Ayres SM. Cardiac catecholamine response during evolving myocardial infarct in man. *Circulation* 1980 (Suppl III);62:III-81. (abstract)], and β-blockers [Mueller HS, Ayres SM. Propranolol decreases sympathetic nervous activity reflected by plasma catecholamines during evolution of myocardial infarction in man. *J Clin Invest* 1980;**65**:338-346.] have long been known to be able to reduce catecholamine levels during acute myocardial infarction. Ischaemic heart disease is the most common cause of developing CHF.

When heart failure has fully developed it is then difficult to establish what exactly induces neurohormonal activation, as both the underlying disease process itself and the medication contribute to the complex hormonal alterations. Measurements in untreated patients have revealed that the sympathetic system is activated (raised catecholamine levels), but that in contrast the renin-angiotensin system is usually not activated [Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang CS, Kubo SH, Rudin-Toretsky E, Yusuf S. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart

failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). *Circulation* 1990;**82**:1724-1729; Remes J, Tikkanen I, Fyhrquist F, Pyorala K. Neuroendocrine activity in untreated heart failure. *Br Heart J* 1991;**65**:249-255.]. The initial sensor to activate these alterations remains unclear, but it is known that in the absence of a neurohormonal body response the blood pressure would fall, ie tissue blood perfusion would be insufficient [Harris P. Congestive cardiac failure: central role of the arterial blood pressure. *Br Heart J* 1987; **58**:190-203.]. Therefore the initial triggers of neurohormonal activation in heart failure could be baroreceptors in the heart and aorta. When heart failure progresses other mechanisms may gain more importance. The baroreflex responses are blunted in stable chronic heart failure, whereas the peripheral and central chemoreflex sensitivity [Pomikowski P, Chua TP, Piepoli M, Ondusova D, Webb-Peploe K, Harrington D, Anker SD, Volterrani M, Colombo R, Mazzuero G, Giordano A, Coats AJ. Augmented peripheral chemosensitivity as a potential input to baroreflex impairment and autonomic imbalance in chronic heat failure. *Circulation* 1997 Oct 21;**96**(8):2586-2594; Chua TP, Clark AL, Amadi AA, Coats AJ. Relation between chemosensitivity and the ventilatory response to exercise in chronic heart failure. *J Am Coll Cardiol* 1996;**27**:650-657.] as well as the metabo-ergoreceptor reflex (afferents sensitive to skeletal muscle work load) [Piepoli M, Clark AL, Volterrani M, Adamopoulos S, Sleight P, Coats AJ. Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: effects of physical training. *Circulation* 1996 Mar 1; **93**(5):940-952.] deliver a strong sympathetic nervous input that may finally also lead to chronically raised catecholamine levels in sever

chronic heart failure.

Catecholamines and weight loss in CHF patients

Only recently, we have documented [Anker SD, Chua TP, Swan JW, Ponikowski P, Harrington D, Kox WJ, Poole-Wilson PA, Coats AJS. Hormonal changes and catabolic/anabolic imbalance in chronic heart failure: The importance for cardiac cachexia. *Circulation* 1997;96:526-534.] that, when considering the conventional disease severity markers peak oxygen consumption, left ventricular ejection fraction (LVEF), and NYHA class, none of these markers very strongly related to resting noradrenaline and adrenaline levels. However, the presence of cardiac cachexia, ie significant non-intentional non-oedematous weight loss (>7.5% of the previous normal weight), related closely to the presence of raised catecholamine levels. Non-cachectic patients with CHF did on average not have elevated catecholamine levels.

Catecholamines can alter the metabolic status of the body, ie they can contribute to increased metabolic rates that may finally lead to a catabolic status and weight loss. This has never been considered to be a basic mechanism for body wasting in human disease in general.

Catecholamines and weight loss in wasting disorders

We have studied a variety of other cachectic conditions - for instance due to AIDS, liver cirrhosis, chronic obstructive pulmonary disease, chronic renal failure, chronic infections (like pneumonia) and cancer - and we

have found activation of the SNS as evidenced by elevated plasma noradrenaline levels (mean plasma levels were clearly above the upper limit of the normal range, see Figures 1 and 2). This is not dependent on any specific aetiology for the cachectic disorder, in fact we find elevated noradrenaline plasma levels (ie SNS activity) also in cases of idiopathic cachexia, ie cachexia of unknown origin. Nevertheless, we find the activation of the SNS to be specific for cachectic disorders, as it is not seen in patients with a similar degree of weight loss consequent upon malnutrition.

Method to measure noradrenaline:

Blood samples were collected after supine rest of at least 10 minutes. An antecubital polyethylene catheter was inserted and 10 ml of venous blood were drawn. After immediate centrifugation aliquots (EDTA plasma sample) were stored at -70°C until analysis. Noradrenaline was measured by reverse-phase high pressure liquid chromatography (HPLC) with electrochemical detection. The detectable limit was: 0.2 nmol/l. The within batch coefficient of variance of repeated measures is less than 5%, the between batch coefficient of variance for repeated measures is 9%. The upper limit of normal for subjects (mean + 2 standard deviations of control group: 3.31 nmol/l).

Example 2: Analysis of aldosterone serum levels in cachectic subjects with chronic wasting disorders

Aldosterone serum levels have been analysed in a number of subjects with these disorders compared to healthy controls, patients with weight loss due to malnutrition (ie no active wasting disease), and CHF patients without cachexia (see Table below and Figure 3). Patients with active wasting disease have on average 2.5 to 13-fold increased aldosterone levels compared to healthy control subjects (their mean: 43.2 ng/ml, upper limit or normal: 81 ng/ml). Patients with weight loss due to malnutrition have normal aldosterone levels. This supports our view that high aldosterone levels are pathophysiologically linked to the presence of chronic active body wasting due, ie cachexia, and that treatment with aldosterone antagonists may be beneficial.

Table: Mean serum aldosterone levels in ng/ml.

Means Table for Aldosterone ng/ml
Effect: Cachexia diag.-Aldost

	Count	Mean	Std. Dev.	Std. Err.
AIDS	4	105.25	124.14	62.07
Cancer	7	163.57	59.59	22.52
cCHF	17	168.18	102.83	24.94
Control	16	43.19	18.87	4.72
Infection	11	184.91	398.17	120.05
Liver/cirrhosis + Cachexia	6	578.17	297.16	121.32
Malnutrition	6	55.50	39.56	16.15
ncCHF	16	98.12	59.07	14.77
Renal failure cachexia	2	456.00	2.83	2.00

cCHF is cachectic CHF and ncCHF is non-cachectic CHF.

We conclude that abnormalities of aldosterone-linked metabolic pathways occur in cachectic disorders independently of the specific aetiology for the cachectic disorder. Nevertheless, we find the alteration of the aldosterone pathway to be specific for cachectic disorders, as it is not seen in patients with a similar degree of weight loss consequent upon malnutrition.

Method to measure aldosterone:

Blood samples were collected after supine rest of at least 10 minutes. An antecubital polyethylene catheter was inserted and 10 ml of venous blood were drawn. After immediate centrifugation aliquots were stored at -70°C until analysis. Aldosterone was measured using a commercially available competitive radioimmunoassay (DPC, Los Angeles, USA, sensitivity 10 ng/ml). This test is a coated tube assay using radio-iodinated tracer. Bound and free phases are separated by decantation. The radioactivity in the bound fractions is measured and a typical standard curve can be generated. The test has a cross-reactivity with spironolactone and aldosterone metabolites of $<1\%$ and a within test coefficient of variance is $<7\%$ and the between test variability is $<10\%$.

Example 3: Endothelin-1 (ET-1), TNF and xanthine oxidase activity

We have previously suggested that the metabo-ergoreceptor reflex (afferents sensitive to skeletal muscle work load) [Piepoli M, Clark AL,

Volterrani M, Adamopoulos S, Sleight P, Coats AJ (1996) "Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: effects of physical training" *Circulation* **93(5)**, 940-952] can deliver a strong sympathetic nervous input that may finally lead to chronically raised catecholamine levels, ie that *via* this mechanism activation of the sympathetic nervous system (SNS) may occur. We have presented data in Example 2 that catecholamine levels are specifically raised in many cachectic syndromes.

The sensitivity of the metabo-ergoreceptor reflex response is determined by the general metabolic status of the musculature, the main determinant of the latter is the blood flow to the musculature, because *via* the blood flow the musculature receives its supply of oxygen and nutrients.

It is a characteristic of cachectic patients with CHF to have a poor peripheral blood flow [Anker SD, Swan JW, Volterrani M, Chua TP, Clark AL, Poole-Wilson PA, Coats AJS (1997) "The influence of muscle mass, strength, fatiguability and blood flow on exercise capacity in cachectic and non-cachectic patients with chronic heart failure" *Europ Heart J* **18**, 259-269]. We have previously published that high uric acid levels [Anker SD, Leyva F, Poole-Wilson, Kox WJ, Stevenson JC, AJS Coates (1997) "Relationship between serum uric acid and lower limb blood flow in patients with chronic heart failure" *Heart* **78**, 39-43] and TNFα [Anker SD, Volterrani M, Egerer KR, Felton CV, Kox WJ, Poole-Wilson PA, Coats AJS (1998) "Tumor necrosis factor - α as a predictor of peak leg blood flow in patients with chronic heart failure" *Q J Med* **91**,

199-203] are very strong correlates of impaired peripheral blood flow in CHF patients. We now propose that treating high TNFα-levels (with TNFα-antibodies or other drugs to reduce biologically active TNF levels – like soluble TNF receptor constructs) and/or high uric acid levels (with xanthine oxidase inhibitors) may improve skeletal muscle blood flow, thereby muscle metabolic status and then metabo-ergoreceptor reflex response, and finally SNS status and the wasting disorder improve.

Another possibility to treat cachexia arises when endothelin-1 (ET-1), the strongest endogenous vasoconstrictive hormone, is considered. Its levels have never been determined in cachectic patients. We present data that ET-1 is significantly highest in cachectic CHF patients ($p<0.05$ vs controls and non-cachectic CHF patients, respectively), although NYHA class and left ventricular ejection fraction (LVEF) were not different between patient groups. Also age was not different between groups. CHF patients without cachexia do not show abnormal ET-1 levels.

Table: Clinical characteristics and endothelin-1 (ET-1) levels in CHF patients with and without cachexia and healthy control subjects.

parameter	**controls n=7**	**non-cachectic CHF n=11**	**cachectic CHF n-12**
age (years)	70±2	66±3	67±3
NYHA class		2.3±0.1	2.7±0.3
LVEF (%)		34±5	30±6
ET-1 (pmol/l)	1.97±0.38	2.22±0.28	2.98±0.20

Although not being bound by any theory a proposed mechanism of action is:

a) inhibition of ET-1 bioactivity by blocking ET-1 receptors, then induction of vasodilation, improvement of muscle blood flow and thereby of metabolic status, then less stimulation of SNS activation, positive effects on cachexia;

b) blocking of TNFα bioactivity, less damage to vasculature and less muscle cell damage directly (inhibition of directly detrimental effects of TNF) and indirectly (inhibition of oxygen free radical generation due to TNF action), thereby improvement of muscle blood flow and muscle cell function and thereby of muscle metabolic status, then less stimulation of SNS activation, positive effects on cachexia and wasting in general;

c) blocking of xanthine oxidase activity, less production of xanthine oxidase derived oxygen free radicals, therefore less damage to vasculature and muscle cells, thereby improvement of muscle blood flow and muscle cell function and thereby of muscle metabolic status, then less stimulation of SNS activation, positive effects on cachexia and wasting in general.

The improved muscle blood flow, muscle cell function and muscle metabolic status believed to be brought about by blocking of TNFα activity is considered to be beneficial in enhancing exercise performance in a healthy patient.

Example 4: Cardiorespiratory reflexes in chronic heart failure (CHF) patients with cardiac cachexia

Cardiac cachexia in patients with chronic heart failure (CHF) predicts very poor prognosis and is linked to neurohormonal activation and an altered balance between catabolism and anabolism (in favour of catabolism).

Impaired sympatho-vagal balance in CHF is important part of neuroendocrine overactivity, is linked to a poor outcome and the underlying mechanisms remain unexplained, but overactive muscle ergoreflex system is one possible stimulus.

Having in mind the neurohormonal changes and high mortality in CHF patients with cardiac cachexia, we hypothesised that in these patients a particularly abnormal pattern of cardiorespiratory reflexes is present. The aim of the study described here was to assess whether impaired reflex control within the cardiorespiratory system (as evidenced by baroreflex inhibition, peripheral chemoreflex overactivity, and abnormal heart rate variability [HRV] patterns) is associated with the presence of cardiac cachexia rather than with conventional markers of CHF severity.

Patients

39 stable CHF patients studied:

all men, age 60 y, NYHA class: II-IV, peak VO_2:17 ml/kg/min, LVEF:24%

Patients divided into 2 groups:

- 13 patients with cardiac cachexia vs 26 non-cachectic CHF patients
- cachectic and noncachectic patients were matched according to age and CHF disease severity

Cardiac cachexia:

non-intentional, non-edematous, documented weight loss >7.5% of the previous normal weight over a period of >6 months, and a BMI (=weight/height^2) <24 kg/m^2 (to exclude obese dieters)

Control Subjects

For the comparison of the results of HRV and baroreflex sensitivity 11 healthy controls (all men, mean age: 60±7 y) were studied.

For the comparison of the results of peripheral chemosensitivity and hormonal measurements data for healthy data for healthy control subjects from the following studies were used:

- peripheral CHEMO (chemoreflex sensitivity): Chua TP *et al* (1995) *Eur J Clin Invest* **25**, 887
- hormonal measurements: Anker SD *et al* (1997) *Circulation* **96**, 526

Methods (1)

1. Evaluation of the cardiorespiratory reflex control

Assessment of the sympatho-vagal control of heart rate

power spectral analysis of HRV derived from 20 minutes recorded the following spectral bands were identified: very low frequency (0.003-0.04Hz, VLF), low frequency (0.05-0.14Hz, LF), and high frequency (0.15-0.40Hz, HF)

Peripheral chemosensitivity evaluation

transient hypoxic method (the ventilatory response to hypoxia using transient inhalations of pure nitrogen)

Methods (2)

Baroreflex sensitivity

phenylephrine method

2. Hormonal measurements

Fasting venous blood samples

collected in the morning (9 and 10 am)

after patients' supine rest of at least 20 min

levels of epinephrine and norepinephrine measured using HPLC (sensitivity 0.1 ng/ml for both)

Results (1)

Table: HRV measures in controls, non-cachectic (ncCHF) and cachectic (cCHF) patients

	Controls	**ncCHF (n=26)**	**cCHF (n=13)**	**p-value**
Mean RR (ms)	1009±133	875±125	790±181	cCHF vs ncCHF NS cCHF vs cont 0.0008 ncCHF vs cont 0.01
TP (In ms^2)	7.1±0.6	6.7±1.2	6.1±0.7	NS
VLF (%TP)	63±12	76±12	85±10	cCHF vs ncCHF 0.07 cCHF vs cont 0.0002 ncCHF vs cont 0.004
LF (In ms^2)	5.6±0.9	4.2±1.4	1.7±1.5	cCHF vs ncCHF <0.0001 cCHF vs cont <0.0001 ncCHF vs cont 0.008
LF (normalised units)	64±19	42±21	15±18	cCHF vs ncCHF 0.002 cCHF vs cont <0.0001 ncCHF vs cont 0.009
HF (In ms^2)	4.7±1.1	4.1±1.3	3.3±0.9	NS

5

Results (2)

Table: Baroreflex sensitivity, peripheral chemosensitivity and hormonal measures in controls, non-cachectic (ncCHF) and cachectic (cCHF) patients

	Controls	ncCHF (n=26)	cCHF (n=13)	p-value
Baroreflex sensitivity (ms/mmHg)	9.2±4.9	5.5±3.5	1.5±1.9	cCHF vs ncCHF 0.04 cCHF vs cont 0.0005 ncCHF vs cont 0.02
Peripheral chemosensitivity (L/min/%SaO_2)	0.29±0.21	0.47±0.20	0.91±0.37	cCHF vs ncCHF <0.0001 cCHF vs cont <0.0001 ncCHF vs cont 0.05
Epinephrine (nmol/L)	0.51±0.16	0.68±0.23	2.46±1.74	cCHF vs ncCHF <0.0001 cCHF vs cont <0.0001 ncCHF vs cont NS
Norepinephrine (nmol/L)	1.94±0.68	2.34±0.16	4.61±3.92	cCHF vs ncCHF 0.02 cCHF vs cont <0.003 ncCHF vs cont NS

Conclusions

1. Patients with chronic heart failure who developed cardiac cachexia demonstrate particularly abnormal reflex control within the cardiovascular and respiratory systems.

2. The nature of the link between this phenomenon and the hormonal changes and the poor prognosis of cachectic CHF patients raises the potential for novel therapeutic strategies targeting the wasting process in cachectic CHF patients by altering the reflex status of patients that could lead to less activation of the sympathetic nervous system and better symptomatic status.

Example 5: Treatment with atenolol

A hypertensive patient presented weighing 85.6 kg. He was treated with Losartan 50 mgs OD, Bendrofluazide 2-5 mgs OD, Doxazosin 1 mg OD and Atenolol, a β-blocker, 50 mgs OD. In 11 months his weight increased to 94.3 kg.

Example 6: Treatment of cachexia patients with an ATII receptor antagonist (Losartan)

We propose that treatment with an ATII receptor antagonist is of benefit for cachectic patients - even if such patients are previously treated with an ACE inhibitor. To exemplify this, we have treated one patient with cachexia due to chronic heart failure (CHF) (age 74 years, male, weight

50.0 kg, height 178 cm, previous weight loss 15.3 kg in 3 years = chronic weight loss) and a second patient with CHF and a muscle myopathy suffering from idiopathic cachexia (age 38 years, male, weight 62 kg, height 180 cm, previous weight loss 11 kg in 1 year = recent weight loss) with Losartan (50 mg once daily) and we have studied clinical status and parameters of body composition, strength and treadmill exercise capacity at baseline and during follow-up. Both patients had evidence of CHF with impaired exercise capacity and impaired left ventricular function (LVEF <40%). Both patients had a good compliance.

Used Methods:

1. Bioelectrical impedance analysis (patient 1 and 2) was performed in the erect position using a body fat analyser (TANITA TBF-305, Tanita Corporation, IL, USA). Lean and fat mass were automatically analysed based on equations supplied and programmed into the machine by the manufacturer. These equations are based upon a comparison with measurements in a healthy population.

2. Dual energy x-ray absorptiometry (DEXA) (patient 1): Whole body DEXA-scans were performed in the Royal Brompton Hospital, London using a Lunar model DPXIQ total body scanner (Lunar Radiation Company, Madison, WI, USA, Lunar system software version 4.3c). The subject was at each time point scanned rectilinearly from head to toe. A scan takes less than 20 min. The mean radiation dose per scan is reported to be about 0.75 μSv [1], about 1/50th of a normal chest x-ray. The DEXA method can be used to obtain from body density analyses values of fat tissue mass, lean tissue mass. The technical details of DEXA, performance and segment demarcation have been described by

Mazess *et al* [2,3]. The error of lean tissue measurements is <2% and of fat tissue measurements <5% [4].

3. Treadmill exercise test (patient 1 and 2): The patients underwent symptom limited treadmill exercise testing. A standard Bruce protocol with the addition of a "stage 0" consisting of 3 min at a speed of 1 mile per hour with a 5% gradient was used. The patients breathed through a one-way valve connected to a respiratory mass spectrometer (Amis 2000, Odense, Denmark) and minute ventilation, oxygen consumption and carbon dioxide production were calculated on line every 10 seconds using a standard inert gas dilution technique. Patients were encouraged to exercise to exhaustion. Exercise time and oxygen consumption at peak exercise adjusted for total body weight (peak VO_2 in ml/kg/min) were measured as an index of the exercise capacity.

4. Assessment of quadriceps muscle strength (patient 1 and 2): The subjects were seated in a rigid frame, with the legs hanging freely. An inelastic strap attached the ankle to a pressure transducer. The recording (Multitrace 2, §, Jersey, Channel Islands) from the pressure transducer was used to assess strength and to provide visual feedback to the subject. A plateau of maximum force production indicated that the contraction was maximal. The best of three voluntary contractions on each leg, with a rest period of at least one minute in-between, was taken to represent the maximal voluntary quadriceps muscle strength of the right and left leg, respectively.

Results

Results include a follow-up of 126 days in patient 1 and 83 days in patient 2. Both patients were also studied at intermediate time points. Both

patients improved during treatment by 1 NYHA symptom class. In both patients the exercise capacity improved during the study (exercise time: patient 1 and 2, peak VO_2: patient 2). There was evidence that in both patients quadriceps muscle strength improved in both legs. These clinical benefits were achieved on the background on a weight gain of 4.6 kg in patient 1 (lean and fat tissue gain), and by stopping the process of weight loss and apparently improving the general clinical status and relative muscle performance, ie muscle quality (patient 2). We observed no side effects of treatment.

References for Example 6

1. Fuller NJ, Laskey MA, Elia M. "Assessment of the composition of major body regions by dual-energy x-ray absorptiometry (DEXA), with special reference to limb muscle mass." (1992) *Clinical Physiology* **12**, 253-266.

2. Mazess R, Collick B, Trempe J, Barden H, Hanson J. "Performance evaluation of a dual-energy x-ray bone densiometry." (1989) *Calcif Tissue Int* **44**, 228-232.

3. Mazess RB, Barden H, Bisek JP, Hanson J. "Dual-energy x-ray absorptiometry for total-body and regional bone mineral and soft-tissue composition." (1990) *Am J Clin Nutr* **51**, 1106-1112.

4. Ley CJ, Lees B, Stevenson JC. "Sex- and menopause-associated changes in body fat distribution." (1992) *Am J Clin Nutr* **55**, 950-954.

Example 7: Elevation of plasma ATII levels in cachectic patients

AT II can directly and indirectly contribute to the development of body wasting. Firstly, AT II can directly induce apoptosis, ie programmed cell death. Secondly, elevated AT II could on the tissue level down-regulate local production of insulin-like growth factor-I (IGF-I). IGF-I is known to be a major factor protecting against apoptosis and it is itsellf strongly protein anabolic.

The detrimental effects of angiotensin II and aldosterone are similar, nevertheless these adverse effects may at least in part be independent of each other. For instance, aldosterone is known to independently reduce magnesium levels by increasing urinary magnesium output, hence magnesium depletion is a prominent feature of many CHF patients (Rahman *et al* (1992) *Scot. Med. J.* **37**, 157-158).

We have studied a variety of cachectic conditions - for instance due to chronic heart failure, AIDS, liver cirrhosis, and cancer - and we have found evidence for elevated plasma AT II levels (mean AT II plasma levels were clearly above the upper limit of the normal range, see Figure 4). This is not dependent on any specific aetiology for the cachectic disorder, in fact we find elevated AT II plasma levels also in cases of idiopathic cachexia, ie cachexia of unknown origin. Nevertheless, we find the elevation of AT II plasma levels to be specific for cachectic disorders, as it is not seen in patients with a similar degree of weight loss consequent upon malnutrition.

Method to measure AT II:

Blood samples were collected after supine rest of at least 10 minutes. An antecubital polyethylene catheter was inserted and 10 ml of venous blood were drawn. After immediate centrifugation aliquots (EDTA plasma sample) were stored at -70°C until analysis. Angiotensin II was measured using a commercially available radioimmunoassay (IBL, Hamburg, Germany, sensitivity 1.5 pg/ml). After extraction of the plasma samples, AT II is assayed by a competitive radioimmunoassay. This radioimmunoassay is using a rabbit anti-AT II antiserum and a radio-iodinated AT II tracer. Bound and free phases are separated by a second antibody bound to solid phase particles, followed by a centrifugation step. The radioactivity in the bound fractions is measured and a typical standard curve can be generated. The test has a cross-reactivity with AT I of <0.1% and a within and between run reproducibility between 3.9 and 8.6%. The reference range for healthy subjects is 20 to 40 pg/ml.

Example 8

The SOLVD treatment study [1] was a randomized, double blind, and placebo-controlled trial investigating the effects of enalapril treatment in clinically stable patients with a LVEF of 35% or less and evidence of overt congestive heart failure. The precise details of study organisation, inclusion criteria, run-in period (2 to 7 days) and stabilization period (14 to 17 days), randomisation, treatment titration and follow-up have been reported previously [1]. The current re-analysis is restricted to subjects who participated in the SOLVD treatment trial (n=2569), and who had been free of edema at baseline and had survived for at least 4 months

thereafter (n=2090). For inclusion into the analysis we also required patients to have weight measurements at baseline and from at least one follow-up visit at 4 months or later. A further 8 subjects with missing or invalid values for weight measurements had to be excluded. The final number of subjects included in this report is 2082 (81.04% of the original trial population). The baseline clinical characteristics of these 2082 patients were not significantly different from the characteristics of the total study population.

Of the 2082 patients, 1055 patients were randomised to treatment with enalapril (2.5 to 20mg per pay) and 1027 patients to treatment with placebo. The clinical characteristics of these two groups were also similar at baseline. During follow-up (range 22 to 51 months), and a total of 756 deaths were observed (36.3%). Body weight at baseline and during follow-up was measured per protocol. Body height was not recorded.

Comparison of means between groups was carried out using an unpaired t-test. Comparison of proportions between groups was made by employing the chi-square test. With regards to the definition of the presence of cachexia different, a priori suggested, cut-points [2] of 5.0%, 7.5%, 10.0% and 15.0% weight loss were considered. To address the question of whether or not ACE inhibitors influence the risk of first occurrence of cachexia, we plotted the cumulative incidence of cachexia in the two treatment groups, and analysed it employing the log-rank statistic [3]. In the analysis of first occurrence of cardiac cachexia, at any given follow-up visit, absence of information on cardiac cachexia (ie weight not documented at this visit) is treated as censored. The effect of cardiac cachexia on survival is assessed using Cox proportional hazard analysis

[2]. For these analyses cardiac cachexia is treated as a time-dependent covariate. The assessment of cardiac cachexia at 4, 8, and 12 months was used in the analysis. These are the time points in the follow-up period with relatively high proportion of complete information on cachexia status. In the database, information on cachexia status is very sparse towards the end of follow-up, which makes it difficult to assess cardiac cachexia as "truly" time-dependent.

The primary analysis was intention-to-treat. Statistical significance is claimed at a computed p-value <0.05 (two-sided testing). Estimates of effects are provided along with their 95% confidence intervals. Results are adjusted for a priori identified prognostic factors such as age, gender, NYHA functional class, LVEF (_25% or >25%), and treatment status (enalapril vs placebo, in the case of assessing the effect of cardiac cachexia on survival).

Of the 2082 CHF patients in this study, 657 (31.6%) developed 7.5% weight loss during follow-up. The cumulative frequency of cardiac cachexia increased continuously over time. The frequency of 7.5% weight loss (cross-sectional) at 1 year was 8.5% and it increased to 15.5% (2 years), and 17.2% (3 years). At baseline patients who developed cardiac cachexia with 7.5% weight loss during follow-up were 1.3 years older (mean 61.2 vs 59.9, $p<0.01$), had 2.7 kg higher weight (mean 80.5 vs 77.8 kg, $p<0.001$), and they were slightly more frequently treated with diuretics (87.2 vs 82.6%, $p<0.01$). Of the patients in this study, 375 (18.0%) were female. Female CHF patients developed cardiac cachexia more frequently (39.5% vs 29.8% in males for 7.5% weight loss, $p<0.001$). Otherwise the baseline clinical characteristics,

particularly with regards to NYHA class, LVEF, and disease etiology, of patients who developed cardiac cachexia and those who did not were similar. The following clinical characteristics at baseline were independently related to the subsequent development of cardiac cachexia: age (RR , $p<0.001$), NYHA class (), LVEF, and treatment.

The development of cardiac cachexia was closely related to subsequently impaired survival. All a priori identified competitive cut-points for cardiac cachexia were related to impaired survival - independent of the effects of age, gender, NYHA class, LVEF, and treatment allocation. Of the 756 deaths observed during follow-up, 223 occurred in patients who had been classified as cachectic (7.5% weight loss) at the last visit prior to death, ie 29.5% of deaths in CHF patients occurred with cardiac cachexia being present. Amongst different cut-offs for cardiac cachexia between 5 and 15%, weight loss 6.5% was the strongest predictor of impaired mortality. The crude effect of cachexia (weight loss 6.5%) on survival was highly significant: RR 1.47 (95% confidence interval: 1.27 to 1.70), $p=0.00000017$.

Patients who were allocated to treatment with enalapril had a significantly lower risk of developing cardiac cachexia during follow-up. The crude effect of treatment allocation with enalapril was significantly related to a reduced risk of developing cardiac cachexia: RR 0.81 (95% confidence interval: 0.70 to 0.95), $p=0.0085$. Treatment allocation to enalapril had a significantly beneficial effect on survival independently of the effect of age, gender, NYHA class, and LVEF also in this subset of patients of the SOLVD treatment trial ($p<0.01$). When we adjusted also for the presence of cardiac cachexia (6.5% weight loss) at 4 or 8 months, the

treatment effect remained significant. In patients who developed weight loss 7.5% at any time point, only 10 patients with subsequently recorded weights equal to or higher than the baseline weight were found (enalapril group: 6, placebo: 4).

This work demonstrates that significant weight loss, ie cardiac cachexia, is a frequent event in CHF patients. Weight loss 7.5% occurs in about 1/3 of patients over 3 years. Spontaneous reversal of the weight loss is a very rare event occurring in less than 2% of cases. Cardiac cachexia is closely and independently linked to impaired survival of CHF patients. Treatment with an angiotensin converting enzyme inhibitor, enalapril, in addition to conventional therapy reduced the frequency of the risk of death and the risk of developing cardiac cachexia. Overall, enalapril therapy reduced the risk of developing cardiac cachexia by 19%.

Figure 5 shows that the frequency of developing cardiocachexia over time is lower with enalapril compared to patients treated with placebo.

It can be estimated that treatment with enalapril delayed the development of cardiac cachexia by about 7 months during the first 3 years. Interestingly, from the SOLVD treatment trial [1] it can be estimated that enalapril delayed the occurrence of death events on average by 5.4 months. A precise estimate of the proportion of the survival benefit of enalapril that was mediated through its benefit on the occurrence of body wasting is not possible to quantify, but the results of the statistical analyses show that at least some of the mortality benefit of angiotensin-converting enzyme inhibitors is mediated through the prevention or delay of cardiac cachexia.

Cardiac cachexia forms a distinct metabolic disease developing on the background of heart failure. Prevention of cachexia by treatment with the angiotensin converting enzyme inhibitor, enalapril, may indicate an important mode of action of this drug and may illustrate the importance of metabolic pathways for the progression of heart failure for its optimum therapy.

References for Example 8

1. The SOLVD Investigators. "Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure." (1991) *N Engl J Med* **325**, 293-302.

2. Cox DR. "Regression models and life-tables." (1972) *Journal of the Royal Statistical Society* **B34**, 187-220.

3. Kalbfleisch JD, Prentice RL. "The statistical analysis of failure time data." (1980) New York: John Wiley and Sons Inc.

Example 9: Treatment of a hypertensive patient with weight loss previously

When the patient was assessed on Day 1 (no ACE inhibitor) he weighed 74.6 kg and had no oedema.

Following treatment with an ACE inhibitor for 5 months and 3 weeks he weighed 76.1 kg.

This shows that an ACE inhibitor can increase body weight in

hypertensive patients besides their effect on lowering blood pressure.

A similar result was found in a second patient.

Example 10: The presence of sympathetic nervous system activation and abnormal sympatho-vagal balance in AIDS - related wasting disease

Sympathetic nervous system (SNS) activation and abnormal sympatho-vagal balance is not only present in patients with cardiac cachexia (Example 4), but also in patients with cachexia due to other disease in the absence of heart failure or any other cardiac disease. The assessment of cardiorespiratory reflex control in 19 patients with documented AIDS disease and documented weight loss of $>10\%$ (mean $22.3 \pm 1.7\%$) and body mass index <20 kg/m^2 was compared to 9 non-cachectic AIDS patients.

The table displays the results of power spectral analyses of heart rate variability (HRV, see methods in Example 4). Statistical test: unpaired t-test. P-values are indicated.

Unpaired t-test for BMI in kg/m2
Grouping Variable: cach?AIDS
Hypothesized Difference = 0
Row exclusion: AIDS HRV-adapted 10/99-StV

	Mean Diff.	DF	t-Value	P-Value
cAIDS, ncAIDS	-5.466	26	-7.349	<.0001

Group Info for BMI in kg/m2
Grouping Variable: cach?AIDS
Row exclusion: AIDS HRV-adapted 10/99-StV

	Count	Mean	Variance	Std. Dev.	Std. Err
cAIDS	19	17.410	3.438	1.854	.425
ncAIDS	9	22.875	3.243	1.801	.600

Unpaired t-test for AGE in years
Grouping Variable: cach?AIDS
Hypothesized Difference = 0
Row exclusion: AIDS HRV-adapted 10/99-StV

	Mean Diff.	DF	t-Value	P-Value
cAIDS, ncAIDS	-4.474	26	-1.475	.1522

Group Info for AGE in years
Grouping Variable: cach?AIDS
Row exclusion: AIDS HRV-adapted 10/99-StV

	Count	Mean	Variance	Std. Dev.	Std. Err
cAIDS	19	38.526	58.041	7.618	1.748
ncAIDS	9	43.000	52.000	7.211	2.404

Unpaired t-test for ln HRV-TP (ln ms2)
Grouping Variable: cach?AIDS
Hypothesized Difference = 0
Row exclusion: AIDS HRV-adapted 10/99-StV

	Mean Diff.	DF	t-Value	P-Value
cAIDS, ncAIDS	-.949	26	-1.897	.0690

Group Info for ln HRV-TP (ln ms2)
Grouping Variable: cach?AIDS
Row exclusion: AIDS HRV-adapted 10/99-StV

	Count	Mean	Variance	Std. Dev.	Std. Err
cAIDS	19	5.357	1.230	1.109	.254
ncAIDS	9	6.306	2.200	1.483	.494

From the results can be concluded:

1. Cachectic AIDS patients show abnormal sympatho-vagal balance (low LF regardless of whether analysed in absolute or normalised units) compared to non-cachectic AIDS patients and healthy controls (see data in Example 4). Also overall HRV (total power: TP) was lower in cachectic vs non-cachectic AIDS patients ($p<0.07$). Although HF was not significantly lower in cachectic AIDS patients vs non-cachectic AIDS patients ($p=0.16$), it was much lower than in healthy subjects or heart failure patients (compare with data in Example 4).

2. The link between abnormal sympatho-vagal balance and hormonal/metabolic abnormalities – in cachectic AIDS patients indicates that the treatments that alter such abnormalities as described herein could have favourable effects on the wasting status of these patients and thereby exert overall beneficial effects.

Example 11: Treatment of a cachectic patient with chronic heart failure with an example beta-blocker (carvedilol)

We disclose herein that beta-receptor blockade is of benefit for cachectic patients - even if such patients are previously treated with an ACE inhibitor. To exemplify this, we have treated a patient with cachexia due to chronic heart failure (CHF) with an aetiology of idiopathic dilated cardiomyopathy (age 60 years, male, weight 69.2 kg, height 183 cm, previous weight loss 10.0 kg [11.6%] in 2 years, indicative of chronic weight loss) with Carvedilol (3.125 mg to 12.5mg twice daily). We have studied body weight, clinical status, parameters of treadmill exercise

capacity, and body composition at baseline and during follow-up. The patient had evidence of CHF with impaired exercise capacity and impaired left ventricular function (fractional shortening 17%) and left ventricular dilation (LVEDD 60 mm) at baseline. The patient had good compliance in taking the carvedilol.

Used Methods:

Body composition was studied using bioelectrical impedance analysis in the erect position using a body fat analyser (TANITA TBF-305, Tanita Corporation, IL, USA). Lean and fat mass were automatically analysed based on equations supplied and programmed into the machine by the manufacturer. These equations are based upon a comparison with measurements in a healthy population.

Treadmill exercise testing: The patients underwent symptom limited treadmill exercise testing. A standard Bruce protocol with the addition of a "stage 0" consisting of 3 min at a speed of 1 mile per hour with a 5% gradient was used. The patients breathed through a one-way valve connected to a respiratory mass spectrometer (Amis 2000, Odense, Denmark) and minute ventilation, oxygen consumption and carbon dioxide production were calculated on line every 10 seconds using a standard inert gas dilution technique. Patients were encouraged to exercise to exhaustion. Exercise time and oxygen consumption at peak exercise adjusted for total body weight (peak VO_2 in ml/kg/min) were measured as an index of the exercise capacity.

Result:

The results show that the patient had an improvement in exercise capacity (peak VO_2 increase of 15%) and in respiratory efficiency indicated by an improvement in VE/VCO_2-slope, which decreased by 15.5%. The increase in exercise capacity was associated with an increase in lean muscle tissue (increased by 1.8 kg). The improvement in VE/VCO_2-slope indicates that muscle metabolic status and reflex status may have additionally improved. In this patient body weight increased by 2.1 kg (3.1%), without development of oedema. The patient tolerated the treatment well.

Conclusion:

Beta-blocker treatment was shown to be beneficial in a cachectic patient.

Example 12: Treatment of cachexia patients with an aldosterone antagonist (spironolactone)

We disclose herein that the blockade of the aldosterone pathway is of benefit for cachectic patients - even if such patients are previously treated with an ACE inhibitor. To exemplify this, we have treated a patient with cachexia due to chronic heart failure (CHF) on the background of coronary artery disease (age 76 years, male, weight 76.0 kg, height 182 cm, previous weight loss 10.0 kg [11.6%] in 3 years, indicative of chronic weight loss) with spironolactone (25 mg once daily). We have studied body weight, clinical status and parameters of treadmill exercise capacity

at baseline and during follow-up. The patient had evidence of CHF with impaired exercise capacity and impaired left ventricular ejection fraction (LVEF 34%) and left ventricular end-diastolic dimension (LVEDD 72 mm) at baseline. The patient had good compliance in taking spironolactone.

Used Methods:

Treadmill exercise testing: The patient underwent symptom limited treadmill exercise testing. A standard Bruce protocol was used. The patient breathed through a one-way valve connected to a commercially available respiratory gas analyser (MedGraphics Inc., USA) and minute ventilation and oxygen consumption were recorded on line every 15 seconds. The patient was encouraged to exercise to exhaustion. Exercise time and oxygen consumption at peak exercise adjusted for total body weight (peak VO_2 in ml/kg/min) was measured as an index of the exercise capacity. One day prior to the intended baseline exercise test an additional exercise test was performed to familarise the patient with the test procedure.

Results:

The results show that the patient had a dramatic improvement in exercise capacity (peak VO_2 increase of 79%, exercise time increased by 53%), the symptomatic New York Heart Association functional class (NYHA class) improved from class III symptoms to class II symptoms. We have evidence that in this patient body weight increased by 1.5 kg (2%),

without development of any oedema. We observed no side effects of the treatment. The improvement of exercise capacity and increase in oxygen consumption was achieved on the basis of a stable peak ventilation, ie it can be concluded that also ventilatory efficiency increased.

Conclusion:

It is well known that the peak oxygen consumption of CHF patients most significantly correlates with leg muscle (lean) tissue mass (Anker *et al* (1998) *Am J. Cardiol.* 83, 612-615). The strong increase in peak oxygen consumption is indicative of the weight increase mainly reflecting an increase of leg muscle tissue. Additionally, the increase in ventilatory efficiency indicates improved ventilatory reflex status which, we think, is due to improved muscle metabolic status. Aldosterone antagonist treatment was shown to be beneficial in a cachectic patient.

CLAIMS

1. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of an agent which reduces sympathetic nervous system activity and/or improves cardiovascular reflex status.

2. A method according to Claim 1 wherein the agent which reduces sympathetic nervous system activity is any one or more of the following: a compound which inhibits the effect of aldosterone such as an aldosterone antagonist; a chymase inhibitor; a cathepsin inhibitor; a β receptor blocker; an imidazoline receptor antagonist; a centrally acting α receptor antagonist; a peripherally acting α receptor antagonist; a ganglion blocking agent; a drug that has an effect on cardiovascular reflexes and thereby reduce SNS activity such as an opiate; scopolamine; endothelin receptor antagonist; a xanthine oxidase inhibitor; and erythropoietin.

3. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of a compound which inhibits the effect of aldosterone, such as an aldosterone antagonist.

4. A method according to Claim 3 wherein the compound which inhibits the effect of aldosterone is any one of spironolactone, testolactone, RU40555, RU26752, canrenoate, eplerenone, 3-(17β-hydroxy-3-oxoandrosta-1,4,6,11-tetraen-17α-yl) propionic acid γ lactone, 3-(9-α-fluoro-17β-hydroxy-3-oxo-androsta-4-en-17α-yl) propionic acid γ lactone,

dihydro-spirorenone, spirorenone, 15,16-methylene derivatives of spironolactone, mespirenone and SC9420.

5. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of a chymase inhibitor.

6. A method according to Claim 5 wherein the chymase inhibitor is any one of alendronate, aprotinin and tissue inhibitors of matrix metalloproteinases.

7. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of a cathepsin inhibitor.

8. A method according to Claim 7 wherein the cathepsin B inhibitor is any one of an epxoysuccinyl peptide such as CA-074 or E64-c, stefin A and cystatin C.

9. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of a β receptor blocker.

10. A method according to Claim 9 wherein the β receptor blocker is any one of acebutolol, alprenolol, atenolol, betaxolol, bisoprolol, carteolol, celiprolol, esmolol, labetolol, lavobunolol, metipranolol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, propanolol, sotalol, timolol, nebivolol, carvedilol and bucindolol.

11. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of an imidazoline receptor antagonist.

12. A method according to Claim 11 wherein the imidazoline receptor antagonist is any one of moxonidine, rilmenidine, pentamidine and α-methyl dopa.

13. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of a centrally acting α receptor agonist.

14. A method according to Claim 13 wherein the centrally acting α receptor agonist is clonidine.

15. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of a peripherally acting α receptor antagonist.

16. A method according to Claim 15 wherein the peripherally acting α receptor antagonist is any one of doxazosin, prazosin, terazosin and ipsapirone.

17. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of a ganglion blocking agent.

18. A method according to Claim 17 wherein the ganglion blocking agent is any one of azamethonium, dicolinium, hexamethonium, mecamylamine, pentamethonium, pentolinium, trimetaphan, benzohexonium, hexafluorenium, cypenam, trimethaphan canfosulfonate, tetraethylammonium bromide and synapleg.

19. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of a drug that has an effect on cardiovascular reflexes and thereby reduces SNS activity.

20. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of an opiate.

21. A method according to Claim 20 wherein the opiate is any one of dihydrocodeine, morphine, diamorphine and buprenorphine.

22. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of scopolamine.

23. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of an endothelin receptor antagonist.

24. A method according to Claim 23 wherein the ET-1 receptor antagonist is any one of butenolide, BQ123, BQ-788, A-216546, ABT-

627, IRL3461, LU135252, S-0139, T-0201, PD 142,893, PD 164333, RO 61-1790, PD 156,707, SB 209670, IRL 1038 and WS-7338 B.

25. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of a xanthine oxidase inhibitor.

26. A method according to Claim 25 wherein the xanthine oxidase inhibitor is any one of allopurinol, 7,8-dihydroneopterin, 5,6,7,8-tetrahydrobiopterin, leukopterin, xanthopterin, neopterin, biopterin, 4-amino-6-hydroxypyrazolo[3,4-d]pyrimidine (AHPP) and oxypurinol.

27. A method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of erythropoietin.

28. A method of treating weight loss due to underlying disease in a patient the method comprising electrically stimulating the patient's muscles.

29. A method according to any one of the preceding claims wherein the underlying disease is any one of AIDS, liver cirrhosis, chronic obstructive pulmonary disease with or without emphysema, chronic renal failure, chronic infections, cancer, heart disease including hypertension and chronic heart failure.

30. A method according to any one of Claims 1 to 29 wherein the patient has idiopathic cachexia.

31. A method according to any one of Claims 1 to 29 wherein the underlying disease is chronic heart failure and the patient has cardiac cachexia.

32. Use of a compound as defined in any one of Claims 1 to 36 in the manufacture of a medicament for treating weight loss due to underlying disease.

33. Use according to Claim 32 wherein the underlying disease is as defined in Claim 28.

34. Use of a compound as defined in any one of Claims 1 to 36 in the manufacture of a medicament for treating idiopathic cachexia.

35. A method of treating or preventing weight loss due to the ageing process in a patient the method comprising administering to the patient an effective amount of an agent which reduces sympathetic nervous system activity.

36. A method of treating or preventing weight loss due to the ageing process in a patient the method comprising administering to the patient an effective amount of any one or more of a compound which inhibits the effect of aldosterone such as an aldosterone antagonist; a chymase inhibitor; a cathepsin inhibitor; a β receptor blocker; an imidazoline receptor antagonist; a centrally acting α receptor antagonist; a peripherally acting α receptor antagonist; a ganglion blocking agent; a drug that has an effect on cardiovascular reflexes and thereby reduce SNS activity such as

an opiate, a digitalis alkaloid, scopolamine; an endothelin receptor antagonist; a xanthine oxidase inhibitor; and erythropoietin.

37. A method of treating or preventing weight loss due to the ageing process in a patient the method comprising electrically stimulating the patient's muscles.

38. A method of enhancing exercise performance in a healthy patient the method comprising administering to the individual an effective amount of an agent which reduces sympathetic nervous system activity.

39. A method of enhancing exercise performance in a healthy individual the method comprising administering the individual an effective amount of any one or more of a compound which inhibits the effect of aldosterone such as an aldosterone antagonist; a chymase inhibitor; a cathepsin inhibitor; a β receptor blocker; an imidazoline receptor antagonist; a centrally acting α receptor antagonist; a peripherally acting α receptor antagonist; a ganglion blocking agent; a drug that has an effect on cardiovascular reflexes and thereby reduce SNS activity such as an opiate; a digitalis alkaloid; scopolamine; an anabolic growth factor like growth hormone and insulin-like growth factor-I (IGF-I); an endothelin receptor antagonist; a TNFα antagonist; a xanthine oxidase inhibitor; and erythropoietin.

40. A method of enhancing exercise performance in a healthy patient the method comprising electrically stimulating the patient's muscles.

41. A method of preventing weight loss consequent to a cardiovascular disorder in a patient at risk of heart disease the method comprising administering to the patient an effective amount of any one or more of a compound with an inhibiting effect on aldosterone; a β-receptor blocker; an imidazoline receptor antagonist; a centrally acting α receptor agonist; a peripherally acting α receptor antagonist; and a ganglion blocking agent.

42. Use of a compound as defined in Claim 35 or 36 in the manufacture of a medicament for treating or preventing weight loss due to ageing in a patient.

43. Use of a compound as defined in Claim 38 or 39 in the manufacture of an agent for enhancing exercise performance in a healthy individual.

ANOVA Table for NA result

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Cachexia diag.- NA-figure	11	260.240	23.658	2.650	.0020
Residual	103	825.986	8.019		

Model II estimate of between component variance: 1.706
94 cases were omitted due to missing values.

Means Table for NA result
Effect: Cachexia diag.- NA-figure

	Count	Mean	Std. Dev.	Std. Err.
AIDS	6	5.217	4.801	1.960
cachectic CHF	16	4.870	2.518	.650
Cancer	2	8.368	5.056	3.575
chronic renal failure	2	3.896	4.658	3.315
COPD	14	3.843	2.305	.616
healthy controls	16	1.940	.687	.172
Ideopathic cachexia	2	3.836	3.203	2.265
Infection	8	6.437	8.868	2.844
Livercirrh + Cachexia	6	8.098	5.693	2.324
Malnutrition	8	2.987	1.764	726
more Controls	3	2.373	1.099	634
nc CHF	37	2.684	1.344	.221

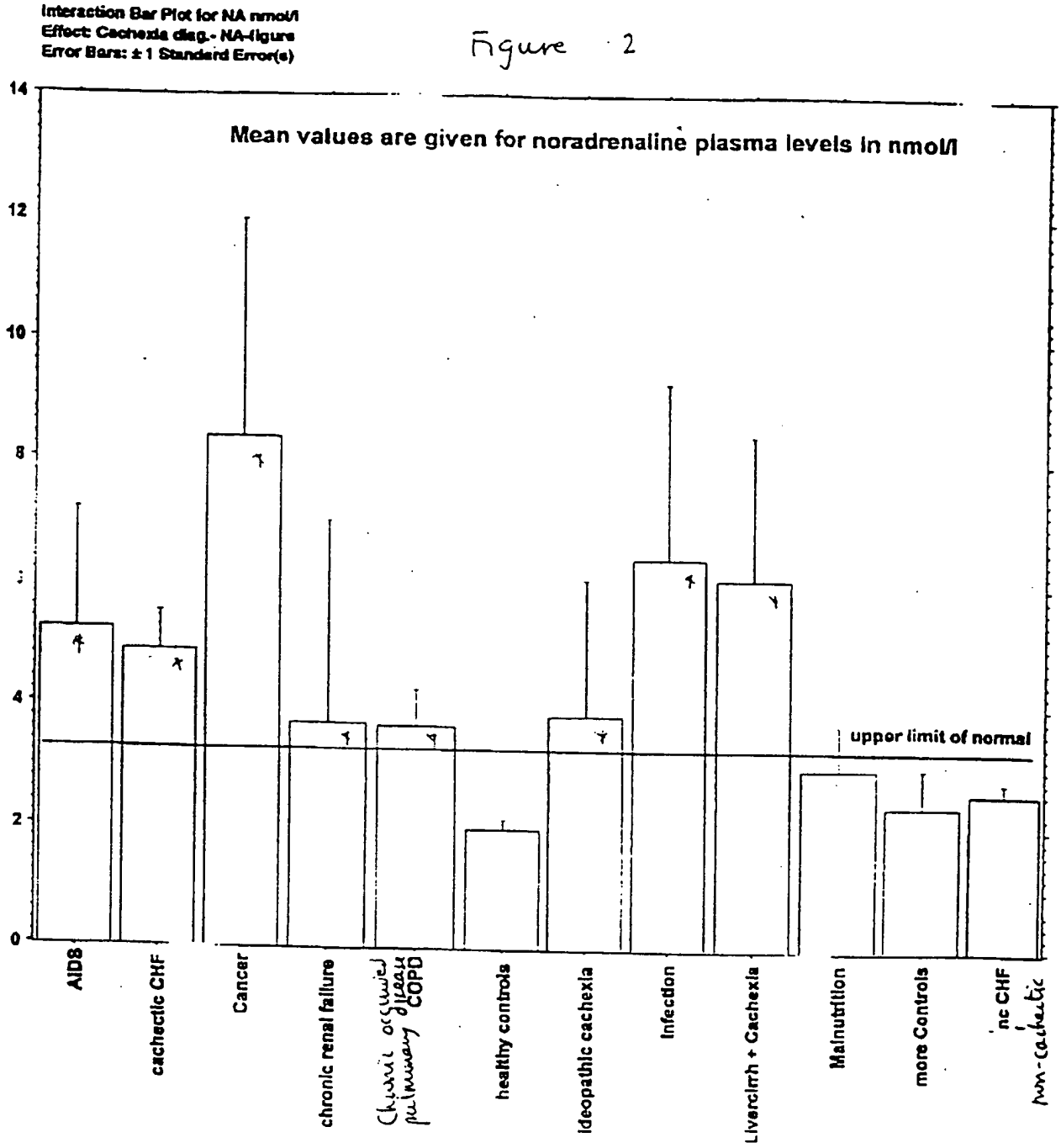
Figure 1

Individual data as summarised in Figure 2

Fisher's PLSD for NA result
Effect: Cachexia diag.- NA-figure
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
AIDS, cachectic CHF	.347	2.718	.8084	
AIDS, Cancer	-3.148	4.586	.1783	
AIDS, chronic renal failure	1.322	4.586	.5119	
AIDS, COPD	1.374	2.740	.2575	
AIDS, healthy controls	3.277	2.689	.0174	S
AIDS, Ideopathic cachexia	1.382	4.586	.5514	
AIDS, Infection	-1.220	3.243	.4572	
AIDS, Livercirrh + Cachexia	-.882	3.243	.6966	
AIDS, Malnutrition	2.230	3.243	.1756	
AIDS, more Controls	2.843	3.971	.1686	
AIDS, nc CHF	2.533	2.472	.0371	S
cachectic CHF, Cancer	-3.495	4.586	.1042	
cachectic CHF, chronic renal failure	1.176	4.586	.5827	
cachectic CHF, COPD	1.227	2.687	.5482	
cachectic CHF, healthy controls	2.930	2.618	.0049	S
cachectic CHF, Ideopathic cachexia	1.035	4.586	.6283	
cachectic CHF, Infection	-1.567	2.713	.3847	
cachectic CHF, Livercirrh + Cachexia	-1.228	2.713	.5719	
cachectic CHF, Malnutrition	1.883	2.713	.1716	
cachectic CHF, more Controls	2.497	3.582	.1663	
cachectic CHF, nc CHF	2.186	1.719	.0096	S
Cancer, chronic renal failure	4.670	5.616	.1022	
Cancer, COPD	4.722	4.246	.0286	S
Cancer, healthy controls	5.425	4.212	.0091	S
Cancer, Infection	1.928	4.586	.4962	
Cancer, Livercirrh + Cachexia	2.267	4.586	.3292	
Cancer, Malnutrition	5.378	4.586	.0220	S
Cancer, more Controls	5.992	5.127	.0224	S
Cancer, nc CHF	5.781	4.077	.0068	S
chronic renal failure, COPD	.052	4.246	.9886	
chronic renal failure, healthy controls	1.755	4.212	.4165	
chronic renal failure, Ideopathic cachexia	-.140	5.616	.9687	
chronic renal failure, Infection	-2.742	4.586	.2394	
chronic renal failure, Livercirrh + Cachexia	-2.403	4.586	.3016	
chronic renal failure, Malnutrition	.708	4.586	.7600	
chronic renal failure, more Controls	1.322	5.127	.6105	
chronic renal failure, nc CHF	1.111	4.077	.5900	
COPD, healthy controls	1.703	2.064	.1035	
COPD, Ideopathic cachexia	-.192	4.246	.9285	
COPD, Infection	-2.794	2.740	.0456	S
COPD, Livercirrh + Cachexia	-2.456	2.740	.0785	
COPD, Malnutrition	.656	2.740	.6386	
COPD, more Controls	1.269	3.573	.4827	
COPD, nc CHF	1.059	1.782	.2582	
healthy controls, Ideopathic cachexia	-1.895	4.212	.3743	
healthy controls, Infection	-4.497	2.689	.0018	S
healthy controls, Livercirrh + Cachexia	-4.158	2.689	.0028	S
healthy controls, Malnutrition	-1.047	2.689	.4418	
healthy controls, more Controls	-.433	3.533	.8083	
healthy controls, nc CHF	-.644	1.680	.4491	
Ideopathic cachexia, Infection	-2.602	4.586	.2631	
Ideopathic cachexia, Livercirrh + Cachexia	-2.263	4.586	.3209	
Ideopathic cachexia, Malnutrition	.648	4.586	.7144	
Ideopathic cachexia, more Controls	1.468	5.127	.5730	
Ideopathic cachexia, nc CHF	1.251	4.077	.5441	
Infection, Livercirrh + Cachexia	.338	3.243	.8368	
Infection, Malnutrition	3.450	3.243	.0373	S
Infection, more Controls	4.069	3.971	.0450	S
Infection, nc CHF	3.853	2.472	.0026	S
Livercirrh + Cachexia, Malnutrition	3.112	3.243	.0596	
Livercirrh + Cachexia, more Controls	3.725	3.971	.0637	
Livercirrh + Cachexia, nc CHF	3.515	2.472	.0054	S
Malnutrition, more Controls	.619	3.971	.7600	
Malnutrition, nc CHF	.403	2.472	.7473	
more Controls, nc CHF	-.210	3.371	.9017	

Figure 2



Chronic wasting disorders show increased activity of SNS as evidenced by increased plasma noradrenaline levels

* All of these cachectic diseases have higher mean plasma noradrenaline levels which are higher than normal

Figure 3

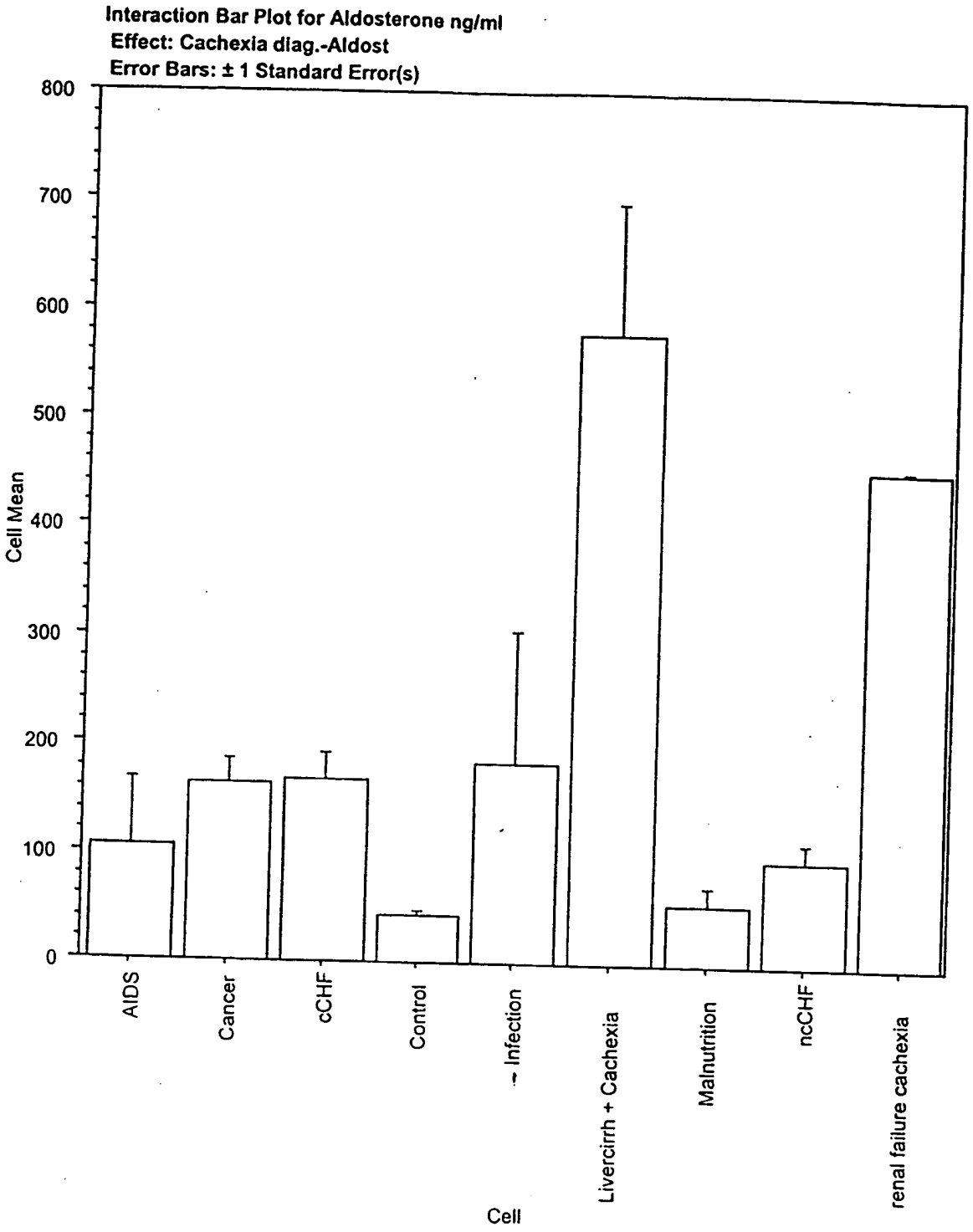
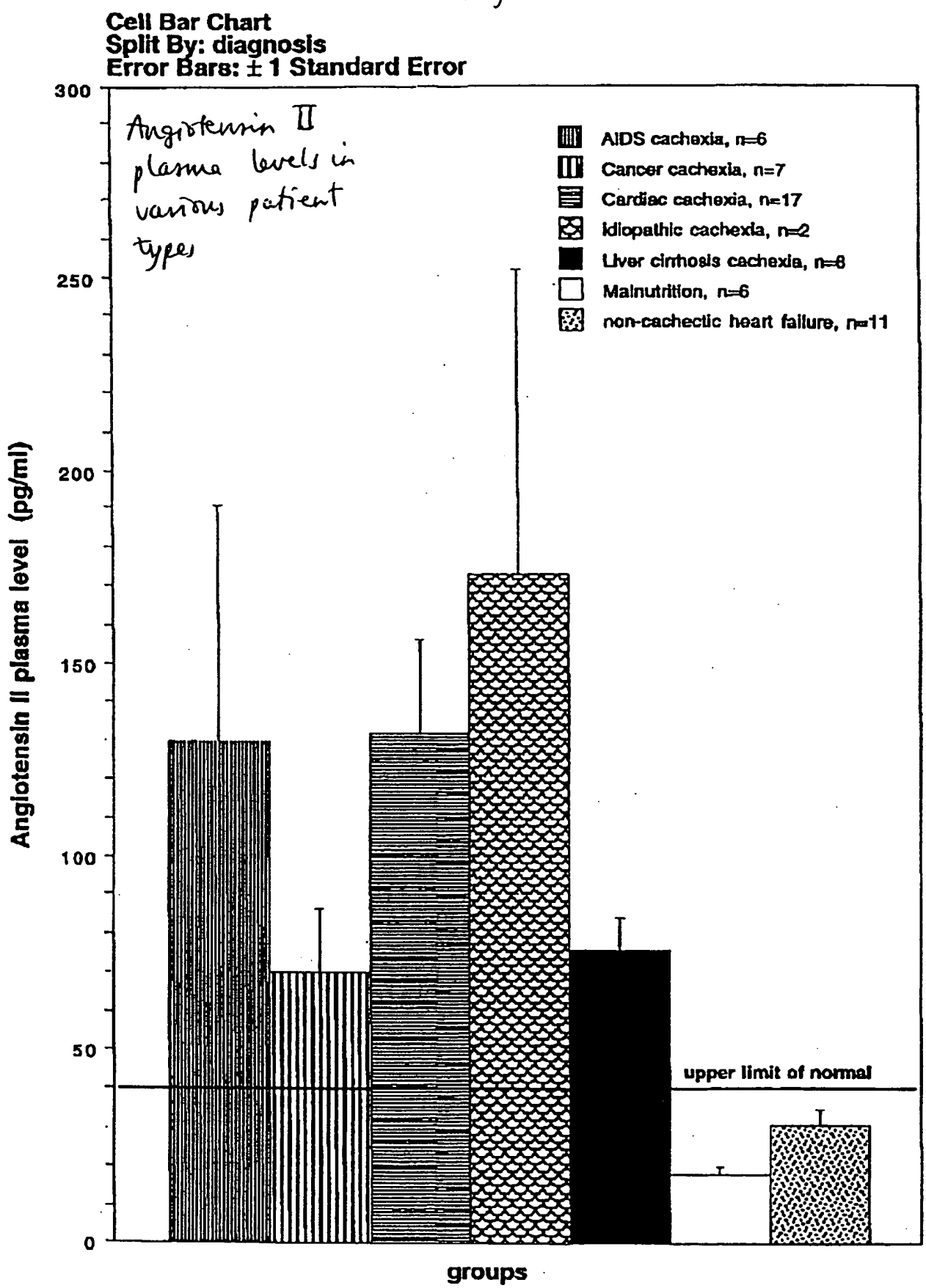


Figure 4



Patients with wasting disease have increased angiotensin II plasma levels

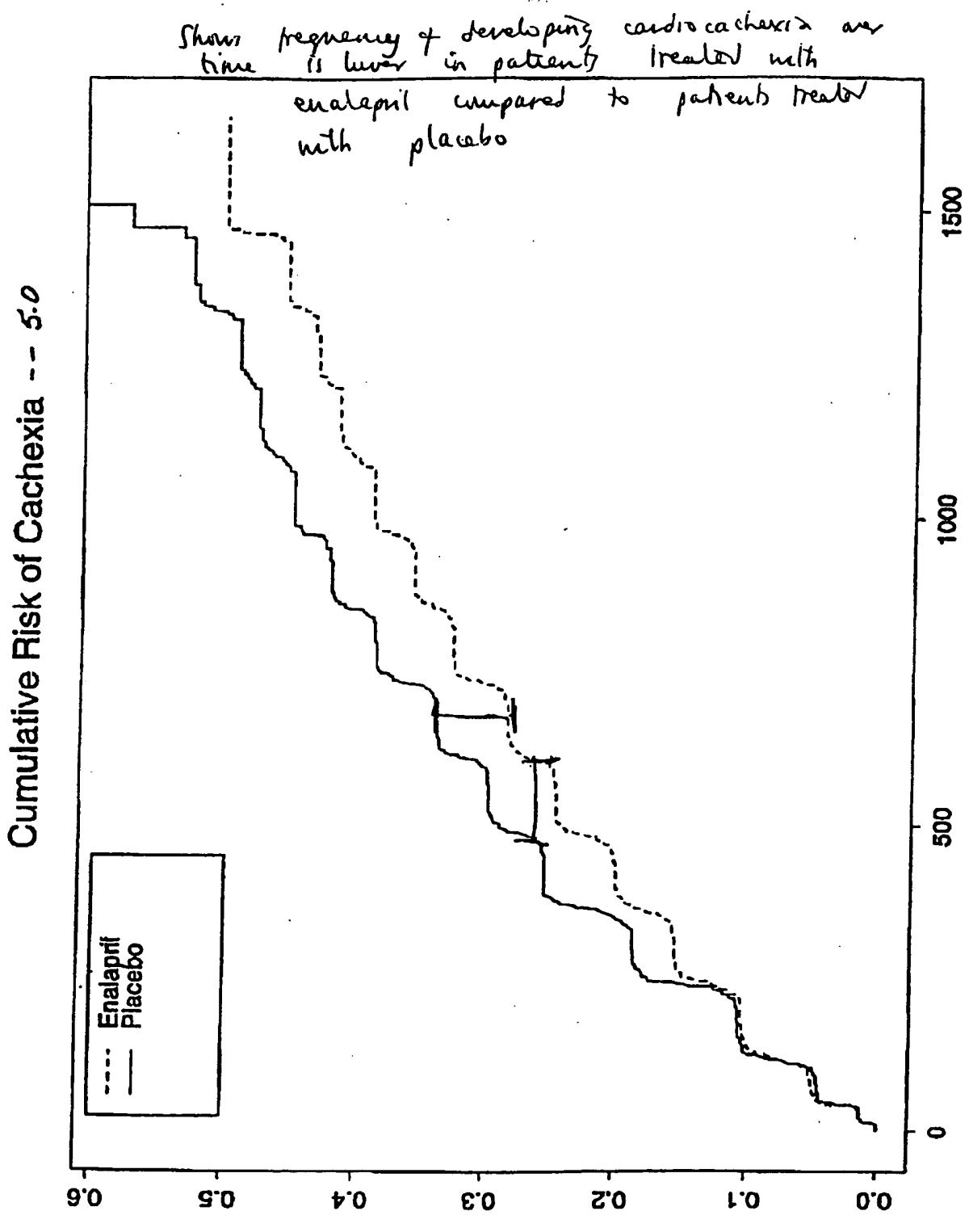

Shows frequency of developing cardiocachexia over time is lower in patients treated with enalapril compared to patients treated with placebo
Cumulative Risk of Cachexia -- 5.0
Enalapril
Placebo
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
500
1000
1500

Figure 5